Data Evaluation Record on the Acute Toxicity of Fluopyram (AE C656948) + Tebuconazole (HWG 1608) SC 400 G to Freshwater Fish- Rainbow Trout (*Oncorhynchus mykiss)*

EPA MRID Number 47567612

Data Requirement: EPA DP Barcode: D386298
EPA Guideline: OPPTS 850.1075

Test material: FLU+TBZ SC200+200A G **Purity:** 17.1% w/w FLU, 17.5% w/w TBZ
Common name
Chemical name: Fluopyram (AE C656948) and Tebuconazole (HWG 1608)

Primary Reviewer: Stephen Carey, Biologist
EPA/OCSPP/OPP/EFED/ERB6

Signature: 7/27/11
Date: 7/27/11

Secondary Reviewer(s):
{EPA/OECD/PMRA}

Date:

Reference/Submission No.: {.....}

EPA PC Code 080302/128997

CITATION: Dorgerloh, M. 2007. Acute Toxicity of Fluopyram & Tebuconazole SC 400 (200 + 200) G to fish (*Oncorhynchus mykiss*) under static conditions. Unpublished study performed by Bayer CropScience AG, Monheim am Rhein, Germany. Laboratory Project Number: E280 3308-4. Document No. M-293228-01-2. Report ID. EBGMP072. Study sponsored by Bayer CropScience AG, Monheim am Rhein, Germany. Study completed September 24, 2007.

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Executive Summary:

In a 96-h acute toxicity test, rainbow trout (*Oncorhynchus mykiss*) were exposed to Fluopyram & tebuconazole SC 400 (200 + 200) G (formulation containing active ingredients fluopyram with 17.9% w/w and tebuconazole with 17.8% w/w) at nominal concentrations of 0 (negative control), 2.50, 5.00, 10.0, 20.0 and 40 mg product/L. The concentrations of the product were not measured; results are based on nominal concentrations. Tebuconazole was not measured; however, fluopyram was measured for verification of the exposed test concentrations. The test conditions met all validity criteria, given by the mentioned guidelines. Accompanying chemical analysis of fluopyram revealed recoveries slightly above the nominal concentrations (at day 0, the percentage recovery ranged from 112 to 121%). The recoveries of the aged concentrations (day 1, 2 and 4) demonstrated the stability of the test item during the exposure period. In the controls no mortalities or sub-lethal findings were observed. In all test levels ≥ 5.00 mg product/L behavioral changes such as remaining for unusually long periods on the bottom of the aquarium and labored respiration were observed during the entire exposure period.

Based on nominal concentrations, the 96 h - LC_{50} was calculated by probit analysis to be 21.7 mg product/L (C.I.95%: 17.3 – 27.1 mg/L). The NOAEC (highest concentration without sub-lethal effects) is considered to be 2.50 mg product/L. The 96 h – NOAEC (mortality) is considered to be 10 mg product/L.

The toxicity study is scientifically sound but does not satisfy EPA guideline requirement for an acute freshwater fish toxicity study with rainbow trout. The study is classified as supplemental since the test concentrations of the product were not measured.

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I) Material and Methods

Guideline Followed: EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985), OPPTS 850.1075 (Public Draft, 1996), Directive 92/69/EEC, C.1 (1992), OECD No. 203 (rev.1992)

Compliance: The study was conducted in compliance with, and satisfies the requirements of:

- OECD Principles of GLP (1982)
- Annex 1 ChemG Principles of GLP (2002)
- US EPA 40 CFR Part 160
- GLP standards of JMAFF.

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. Materials

1. Test material:	Fluopyram (AE C656948) + tebuconazole (HWG 1608) SC 200 + 200
Specification No:	102000016375
Batch No:	2007-002120
Purity:	Nominal: 200 g fluopyram/L + 200 g tebuconazol/L Analyzed: 201 g fluopyram /L (17.9 % w/w) and 200 g tebuconazol /L (17.8% w/w)
Visual appearance:	White suspension
Density:	1.123 g/mL

2. Test organism:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per concentration and control
Mean body length:	5.8 ± 0.5 cm (mean ± SD)
Mean body weight:	2.2 ± 0.6 g (mean ± SD)
Source:	Lot F 4 / 07 A, delivered on 2007-03-30, was obtained from and identified by Hatchery Rhönforelle, 36129 Gersfeld, Germany.
Acclimation period:	14 days under 16/8 hour light/dark period

3. Environmental conditions:	
Water quality:	Test water was reconstituted water prepared by adding salt stock solutions to demineralized water (conductivity <0.2 µS/cm). Ionic concentrations and hardness (40-60 mg $CaCO_3$/L)
Temperature:	12.4 to 13.3 °C
Photoperiod:	16 hours light / 8 hours dark
pH:	6.7 to 7.1
O_2 content:	78 – 97 % saturation

Total organic carbon:	<2 mg/L
Particulate matter:	<1 mg/L
Metals:	<1 mg/L
Pesticides:	<0.05 mg/L
Chlorine:	<0.01 mg/L

B. Study design and methods

1. In life dates:	2007-05-21 to 2007-05-25
2. Experimental treatment and observations:	
Test duration:	96 hours
Test system:	Static
Test vessel material:	Glass
Test vessel size:	32 x 36 x 38 cm (l x d x h), total volume 44 L
Test medium volume:	40 L
Test vessel loading:	The biomass loading was 0.55 g fish/L test medium
Number of test levels:	1 water control, 5 treatment concentrations
Applied concentrations:	
Nominal:	Formulation: 0 (control), 2.50, 5.00, 10.0, 20.0 and 40 mg test item/L Fluopyram: 0 (control), 0.45, 0.89, 1.79, 3.58, 7.16 mg a.i./L
Measured (mean):	<0.052 (LOQ, control), 0.54, 1.05, 2.09, 4.27 and 8.39 mg ai/L
Number of replicates:	One replicate of 10 fish per test level
Solvent carrier:	None
Solvent load	-
Reference substance:	N/A

Exposure

The test was performed under a static regime. All solutions were prepared by weighing in an adequate amount of the test item into a glass and transferring the needed amounts of the solutions into the vessels by mixing them with test water. At start of the test, ten fish per treatment group were randomly distributed to the aquaria (total number of 60 fish). Fish were not fed 48 hour prior to and during the test.

3. Observations:

Endpoints and water quality

During the test, fish were observed for mortalities and signs of intoxication (sublethal effects) after 4, 24, 48, 72 and 96 hours. Dissolved oxygen, water temperature and pH values were determined daily in each aquarium, water temperature was additionally measured in the control aquarium and recorded hourly with a data logger. In case of 100% mortality prior to the end of the test, the analytical determinations were made at the respective times. Fluopyram were analyzed in all test levels at 0, 48 and 96 hours

Statistical methods

LC_{50} values and the 95%-confidence intervals were calculated every 24-hour using ToxRat® version 2.09, which estimated the LC_{50} using one of three statistical techniques: moving average, logit or probit. The appropriate method was determined according to the data characteristics.

Analytical verification
For verification of the aspired exposure concentrations, content of the active substance AE C656948 was analytically determined. The other active ingredient tebuconazole was not analyzed since it is present in the formulated product in a fixed ratio to the analyzed component. Analytical determinations of fluopyram were made by HPLC-UV. The LOQ is 5 µg/L and the LOD 1.7 µg/L. Fluopyram concentrations were analyzed in the test medium at the beginning (Day 0), 48h and at test termination (96h).

II) Results and Discussion

Findings and observations

Analytical data:
Accompanying chemical analysis of fluopyram (in water via HPLC-UV) revealed recoveries slightly above the nominal concentrations (at day 0, the percentage recovery ranged from 112 to 121%). The recoveries of the aged concentrations (day 1, 2 and 4) demonstrated the stability of the test item during the exposure period. The mean recoveries in the treatment levels (day 0 to day 4) ranged between 111 to 119%.

Behavioral effects and mortality:
No sub-lethal effects were noted in the control or in the treatment level of 2.5 mg product/L throughout the definitive exposure period. In all other treatment levels above 2.5 mg product/L nominal some individuals showed symptoms after 96 h such as remaining for unusually long periods on the bottom of the aquarium and labored respiration (data not presented). Summaries of cumulative mortality and sub-lethal effects are presented in **Tables 1 and 2**, respectively, below.

Table 1: Reported mortality effects on rainbow trout (*O. mykiss*) exposed to Fluopyram + tebuconazole SC 200 + 200.

Nominal concentrations [mg product/L]	Mean measured concentration [mg fluopyram/L]	Cumulative mortality (%)			
		24 hours	48 hours	72 hours	96 hours
Control	<0.1006 (LOQ)	0	0	0	0
2.50	1.31	0	0	0	0
5.00	3.31	0	0	0	0
10.0	8.28	0	0	0	0
20.0	20.3	0	20	30	30
40.0	46.6	100	100	100	100

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Table 1: Reported mortality effects on rainbow trout (*O. mykiss*) exposed to Fluopyram + tebuconazole SC 200 + 200.

Nominal concentrations [mg product/L]	Mean measured concentration [mg fluopyram/L]	Cumulative mortality (%)			
		24 hours	48 hours	72 hours	96 hours
NOAEC: 10 mg product/L (nominal)					
LC_{50} (96 h): 21.7 mg Fluopyram + tebuconazole SC 200 + 200 (nominal)					

Table 2: Reported Sub-lethal Effects on Rainbow Trout (*O. mykiss*) Exposed to Fluopyram + Tebuconazole SC 200 + 200.

Nominal concentrations [mg product/L]	Mean measured concentration [mg fluopyram/L]	Cumulative Sub-lethal Effects (%)			
		24 hours	48 hours	72 hours	96 hours
Control	<0.1006 (LOQ)	0	0	0	0
2.50	1.31	0	0	0	0
5.00	3.31	20 (BO, AT)	40 (BO, DF)	40 (BO, DF, AT)	60 (BO, AT)
10.0	8.28	50 (BO, AT, DF)	80 (BO, DF, AT)	80 (BO, DF, AT)	100 (BO, AT, DF, V)
20.0	20.3	50 (BO, AT, DF, SD)	80 (OB, AT, DF, V, TS) 20 (TF)	70 (BO, SR, BA, DF, AT) 30 (TF)	70 (BO, SR, AT, DF, KR) 30 (TF)
40.0	46.6	100 (BO, SR, AT, DF)	100 (TF)	100 (TF)	100 (TF)
NOAEC: 2.5 mg product/L (nominal)					
EC_{50} (96 h): n.d.					

AT = labored respiration; BO = remain for usually long periods on bottom of the aquarium; DF = dark coloration; KR = convulsions; SR = lie on side or back; TF = dead; TS = loss of equilibrium; V = vertical position; and BA = swollen belly

III) Conclusion of study author:

The test conditions met all validity criteria, given by the mentioned guidelines. Based on nominal concentrations, the 96 h - LC_{50} was calculated by probit analysis to be 21.7 mg product / L (C.I.95%: 17.3 – 27.1 mg product / L). The NOAEC (highest concentration without sublethal effects) is considered to be 2.50 mg product/ L (nominal).

EPA Reviewer's Conclusion:

The test concentrations of the formulation product were not measured. This affects the acceptability of the test.

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The Reviewer agrees with the following endpoints from this acute toxicity study on rainbow trout (*Oncorhynchus mykiss*) exposed to Fluopyram + tebuconazole SC 200 + 200:

LC_{50} (96 h):	21.7 mg product/L nominal	95% C.I.: 17.3 – 27.1 mg product/L
NOAEC (based on mortality):	10.0 mg product/L nominal	
NOAEC (based on sub-lethal effects):	2.50 mg product/L nominal	

REFERENCES

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